METHODOLOGY

HPLC METHOD DEVELOPMENT

Sample preparation is a critical step during analysis. First consideration for developing the analytical methods is to determine the solubility of sample under analysis. Selecting specific solubility of bulk drug in a general solvent like water, methanol, Acetonitrile, Chloroform, hexane, ethanol etc . Mobile phase composition plays a key role in separation of different molecules. Mobile phase composition should be selected such way that it will not degrade the column packaging material and HPLC contact parts. Hence strong acids, bases and halide solution are avoided in mobile phase. Viscosities to be maintain low i.e. less than 0.5 centipoises in order to avoid the higher backpressure. The chemicals and solvents used should be high purity and easily available.

Detector: The selection of the detector is based on the chemical characteristic of the molecules under investigation. Due to the easy availability and as most of the chemical compounds shows absorbance in the UV Visible range, maximum method development starts from UV Visible detector. In some cases the molecules are converted into different form where the resulting compound shows absorbance in UV Visible range. When UV Visible response is not possible than only other types of detectors are considered.

Column : Now a day's intensions is to develop an analytical method where the chromatograms should show maximum separation between different molecules within short run time and to use minimum solvent and protect the environment and cost , short length columns are preferred. The selection of Stationary phase to be selected based on molecule under analysis, generally reverse phases are more preferred e.g. C8, C18.In case of normal phases , most preferred stationary phases is Cyano (Nitrile phase.)

Subsequently the method is fine tuned selecting proper chromatographic Parameters like Flow rate, Column oven Temperature, Wave length, injection volume, tray temperature etc. Each HPLC analytical method should have few of the system suitability parameters

such as Column efficiency, Resolution Factor, Tailing Factor, % RSD of peak area and % RSD of Retention times of multiple injections, Relative Retention Time, Similarity Factor, Capacity Factor, signal to noise ratio are complying to ensure the correctness of analysis .These parameters are based on the outcome of the analytical method validation.

Quality by design Approach (QBD): A systematic approaches to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. During the analytical method development as per QBD approach the variability occurring due to HPLC column, Mobile phase composition, pH etc are evaluated.

ANALYTICAL METHOD VALIDATION

Analytical method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. FDA regulations such as GMP, GLP and GCP and quality standards such as ISO17025 require analytical methods to be validated before and during routine use.

The validation should follow a plan that includes the scope of the method, the method performance characteristics and acceptance limits.

All below parameters and evaluated during validation with predefined protocol and experimental conditions and validation results are documented in a validation report.

SPECIFICITY

ICH defines specificity as “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

PRECISION

ICH defines the precision of an analytical procedure as the closeness of agreement (degree

of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

ACCURACY

ICH defines the accuracy of an analytical procedure as the closeness of agreement between the conventional true value or an accepted reference value and the value found.

Accuracy can also be described as the extent to which test results generated by the method and the true value agree.

LINEARITY

ICH defines linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components.

Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation.

RANGE

ICH defines the range of an analytical procedure as the interval from the upper to the lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The range of an analytical method is the interval from the upper to the lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

LIMIT OF DETECTION (LOD)

ICH defines the detection limit of an individual analytical procedure as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LIMIT OF QUANTITATION (LOQ)

ICH defines the limit of quantitation (LOQ) of an individual analytical procedure as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

RUGGEDNESS

Ruggedness is not addressed in the ICH documents4.5. Its definition has been replaced by reproducibility, which has the same meaning. Ruggedness is defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments,

ROBUSTNESS

ICH defines the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of the procedure's reliability during normal usage.

SOLUTION STABILITY

Solution stability is define storage period in solution form of standard solution by injecting the same stanadard solution after regular time interval.

WORK PLAN:

Literature study.

Literature Survey to find out the availability / non availability of High Performance

Liquid Chromatographic methods, potential problems in available method

Availability of material, Sample, Impurities, Column, chemicals etc.

Development and validation of analytical Method.

Development of analytical method.

Validation of developed analytical method and reporting of the results obtained.

1. Agilent Technologies, Inc.,(**2009),** *HPLC Basic Fundamental of Liquid Chromatography* , 1-35
2. Amit Gupta*, Ram Gaud and S. Ganga,(2010), *" Liquid Chromatographic Method for Determination of Nisoldipine from Pharmaceutical Samples", School of Pharmacy and Technology Management SVKM's NMIMS University, V. L. Mehta Road, Vile Parle (W) Mumbai-56, India,E-journal of Chemistry, 2010, 7(3), 751-756.*
3. Ardrey B,(**2003)**,*Liquid Chromatography –Mass Spectrometry: An introduction* ,10-17
4. A. Sruthi*, N. Thanuja, M. Saisamhita, D. Sudheerkumar, G. Sreekanth I,*"A simple RP-HPLC method for simultaneous estimation of paracetamol and etodolac in tablet dosage form" , Department of Pharmaceutical Analysis, Care College of Pharmacy, Warangal- 506006, Andhra Pradesh, India*
5. Ahuja S, And Rasmussen H.(**2007)**, *HPLC Method Development For Pharmaceuticals*, (8),15-22.
6. Application Notes LCMS, Separation and Quantitation of R and S Enantiomers using normal phase chromatography on an API, *Applied biosystem.*
7. Baldani S., Bhatt K., Mehta R., Shah D. Gandhi T.,(**2008)** ,"RP-HPLC Estimation of Venlafaxine Hydrochloride in Tablet Dosage Forms*", Indian Journal of Pharmaceutical Sciences,*70(1),124-128
8. B. Saidat, Fouziaboudah And M. H. Guermouche.,(**2010),** High Performance Liquid Chromatography Chiral Separation of D, L-Phenylanaline and D, L-Tryptophan with quaternary mobile phase mixture by Copper mixed chelate complexation" , *Analele University din Bucuresti-Chimie, vol 19 no 2, pag. 77-8.*
9. Bakshim.,Singh S.,(**2002)**,"Development of Validated Stability Indicating assay

methods critical review", *Journal of Pharma biomedical analysis*,28,1011-1040.

10. Bhatt D.,Rane S.,(**2011)** "QbD Approach to analytical Method Development and its Validation" , *International Journal Of Pharmaceutical Sciences* , (3.I),179-187.
11. Chang-Chuangua, Yi-Hong Tang, Hai-Hong Hu, Lu-Shan Yu, Hui-Di Jiang,SuZeng,(**2011)**,"Analysis of chiral non-steroidal anti-inflammatory drugs flurbiprofen, ketoprofen and etodolac binding with HSA" *Journal of Pharmaceutical Analysis 2011:1(3):184-190*.
12. Dhiware, Abhijit D.; Gandhi, Santosh V.; Deshpande, Padmanabh B.; Bhavnani, Vandana S.,(**2012)**, *"A simple and sensitive RP-HPLC method for simultaneous estimation of etodolac and thiocolchicoside in combined tablet dosage form", International Journal of Pharmacy &Pharmaceutical Sciences;Oct2012 Supplement, Vol. 4*
13. Dr. Salvatore Caccamese, (**2004)**,"*Direct high-performance liquid chromatography (HPLC) separation of etodolac enantiomers using chiral stationary phases" ,Chirality Volume 5, Issue 3, pages 164-167, 1993.*
14. F.Pehourcq, C. Jarry and Bannwarth. "Chiral resolution of flurbiprofen and ketoprofen enantiomers by HPLC on a glycopeptides type column chiral stationary phase", Biomedical Chromatography2001 May;15(3):217-222.
15. Guo Z, Wangh H, Zhang Y., **(2006)** "Chiral separation of ketoprofen on an achiral C8 column by HPLC using norvancomycin as chiral mobile phase additives".*J Pharm Biomed Anal. 2006 April 11:41(1):310 -4. Epub 2005 Dec 2.*
16. ICH, Harmonised Tripartite Guideline ,(**2005)** ,*Stability Testing of New Drug substances and products* Q1A(R2) , 2 .
17. ICH, Harmonised Tripartite Guideline, (**2005)**, *Validation of Analytical Procedures : Text and Methodology* Q2(R1), 1-13
18. Jing-min SHI, Shun-guo Lai, Chang-jiang XU, Geng-li DUAN, Duan Li, *(2004)," Pharmacokinetic difference between S-(+)- and R-(-)-etodolac in rats", Department of Pharmacology, Department of Pharmaceutical Analysis, School of pharmacy, Fudan University, Shanghai 200032, China,Acta Pharmacol Sin 2004 Aug, 25 (8) : 996-999.*

19. Jing Y ,Ya-Qin S, Zhuo-Rongli,Shao-Hong J,**(2005),** "Development of RP-HPLC method for screening potentially counterfeit ant diabetic drugs" *J Chromatogr B AnalytTechnol Biomed Life Sci*B 853,254-259.
20. John A. A., **1997**, *Chromatographic Analysis of Pharmaceuticals* , Second Edition.
21. Katz E, Ogan And ScootR.P.W, (**1984)**" LC column Design" *J Chromatgr*.289:65.
22. Khan M.A, Sinha S, Vartak S, Bharatiya A, Kumar S **(2005),** "LC determination of Glimeperide and its related impurities", *J Pharm Biomed Anal39*,928-943.
23. Lehr K ,Damm P , (**1990),** Chromatography 526:497-505.
24. Lakshmi K.S, Rajesh T,Sharma S,**(2009)**,"Determination of Pioglitazone and Glimeperide in pharmaceutical formulations and Rat plasma by RP-LC*". International Journal of PharmTech 1,* 496-499.
25. LadN.R,Bhoirc,Sundaresan M, **(2003)**, "Concurrent assay of Metformin and Glimeperide in Tablets using RP-HPLC with wavelength programming". *Journal of Pharmaceutical Sciences 65*,650-653.
26. Lloyd S, Kirkland J.J., Glajch J.,(**1997)**, *Practical HPLC Method Development* , Second Edition , 685-712.
27. M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. Perez-Maseda,"Chiral and nonchiral determination of ketoprofen in pharmaceuticals by capillary zone electrophoresis*". Journal of Chromatography A, Volume 799, Issues 1-2, 13 March 1998, Pages 301-307.*
28. Manikandan Krishanan*,Santhana Laxmi Karunanidhi, Gayathri Sola, Akshitha.Y, (2013),*"Stability indicating HPLC Method for the estimation of Cinacalcet Hydrochloride API", Department of Pharmaceutical analysis, SRM College of Pharmacy, SRM University, Kattankulathur, Tamilnadu 603203, India, Indian journal of research in Pharmacy and Biotechnology, Volume 1 (3), May-June 2013,Page 346-350.*
29. M. Senthil Raja*, Shan. S.H., P. Perumal, and M.T.S. Moorthy,(2010), *"RP-HPLC method development and validation for the simultanious estimation of Azithromycin and Ambroxol Hydrochloride in tablets", Department of pharmaceutical analysis, J.K.K. Nataraja college of pharmacy, Komarapalaym 638 183, Tamil Nadu, India,*

International Journal of pharmaTech Research, Vol.2, No.1, pp 36-39, Jan-Mar 2010.

30. Nandini R. Pai*, Deepnandan S. Dubhashi, (2010), *" Development of stability indicating, validated HPLC method for quantitative determination of Aripiprazole and its impurities", Department of organic chemistry, D.G. Ruparel College, Senapati Bapat Marg, Mahim (West), Mumbai, India,Scholars Research Library,Der Pharmacia Lettre, 2010, 2(4):1-10.*
31. P. Balan, I. Carolinnimila, M. Lakshmi Prasanna, M. Vanaja Rani, S. Rajasekar.(2011), "*RP-HPLC Method Development and Validation of Etodolac and Paracetamol in Combined Dosage Form," Asian J. Research Chem. Vol. 4(7): July, 2011, 1073-1076 06/2011; Vol. 4 (7)(july,2011):1073-1076.*
32. Phanthanh Dung, Tran Quoctrungandkyeong Ho Kim**(2009)**, "Praparative Resolution of Etodolac Enantiomers by Preferential Crystallization method". *Arch pharm Res Vol 32, No 10, 1425-1431 2009.*
33. P. Ravishankar*, G. DevalaRao,(2013), *"A novel Validated RP-HPLC method for the determination of anastrozole in bulk and pharmaceutical tablet dosage forms", Department of Pharmaceutical Analysis and quality Assurance, Vignan Pharmacy college, Vadlamudi, Guntur, Andhra Pradesh, India, Scholars, Research Library, Der Pharma Chemica, 2013, 5(3): 51-62.*
34. Prameela Rani.A*, *Bala Sekaran. C,(2009), "Development of HPLC method for the determination of Olanzapine in bulk and dosage forms", Department of Pharmaceutics, K.V.S.R. Siddhartha college of pharmaceutical sciences, Vijayawada- 520 010, International Journal of Pharma Tech Research , Vol.1, No.3, pp 654-657,July-Sept 2009.*
35. Radisavljevic M,**(2001)**, Sge ,Technical Article : *How do I Develop an HPLC Method* , 1-2.
36. *Prasenjit Mondal** and Battula Neeraja, (2013), " Development of new simple and economic validated RP-HPLC method for the determination of Famciclovir in bulk and tablet dosage", Department of pharmaceutical analysis, Vaageswari college of pharmacy, Karimnagar, Andhra Pradesh, India,Scholars Research Library, Der Pharmacia Lettre, 2013, 5 (4) :320-325.

37. Roston A., Shoupr. ,And Kissinger P.T,(**1982),**"Liquid Chromatography/ Electrochemistry Thin layer multiple electrode detection "*Anal.Chem*,54,1417A.
38. Raghadhommoss, Hind Elzein, Samerhaidar.,(**2011)**,"Determination of Leocitrazineconfigurational stability in tablet using chiral HPLC method",*International Journal of pharmacy and pharmaceutical science",Vol 3, Suppl 2, 2011.*
39. R.V.S Nirogi, S. Kota, B. G. Peruri, V.N. Kandikere And K. Mudigonda**(2006),** "Chiral High Performance Liquid Chromatographic for Enantioselective analysis of Zaltoprofen " *ActaChromatographia, No. 17, 2006.*
40. Srinivas Vishnumulaka, Narashimha Rao Medicherla, Allam Appa Rao and G Edela Srinubabu*, (2008), *"Development and validation of LC Method for the Determination of Famciclovir in pharmaceutical Formulation Using an experimental design", Center for Biotechnology, College of engineering, Andhra University, Visakhapatnam – 530003, India*, E-Journal of chemistry, Vol. 5, No. 1, pp. 58-67, January 2008.
41. Suya Sun, Youzhijia, NingZeng, Famei Li.,(**2006)**,"Chiral Ligand Exchange Chromatography for separation of three Sterioisomers of octahydroindole-2-carbohylic acid", *Chromatographia,* April 2006, Volume 63, Issue 7-8,331-335.
42. Snyder R., Kirkland Jj, GlajchlJi.,(**1988)**, *Practical HPLC Method Development* , John Wiley and Sons, New York;3:2, 2-21.
43. Shrivastavaa.,Nagori B.,(**2010)**,"Stability Indicating High Performance Liquid Chromatography Method for the Estimation of Artemether in Capsule Dosage Forms", *Journal of Young Pharm*.,2(1),79-84.
44. Shibukawa A, Terakita A, He JY, Nakagawa T. "High-performance frontal analysis high-performance liquid chromatographic system for stereoselective determination of unbound ketoprofen enantiomers in plasma after direct sample injection." J. Pharm. Sci.81(1992):710-715
45. Syed Nizamuddin*,K.C. Prashanth, T. Shekshavali, B.K. Sridhar and S. Appala Raju, (2009),*"RP-HPLC method for the estimation of famciclovir in bulk and pharmaceutical Dosage form", Department of Pharmaceutical Analysis, National college of Pharmacy, Shimoga 577 201 (K.S.) India, Int. J. Chem. Sci. : 7(I), 2009, 9-15.*
46. United State Pharmacopeia,(**2011)**, <621>*Chromatography*. 34.

47. USFDA, Code Of Federal Regulation,**(2011),***Part 211–Current Good Manufacturing Practice for Finished Pharmaceuticals* , Vol.4
48. USFDA, Centre For Drug Evaluation And Research, Reviewers Guidance,(**1994)** , *Validation of Chromatographic Methods* , 1-29.
49. V.P Rane And D.B Shinde,(**2008)**, "Development and Validation of Chiral LC method for Enantiomeric separation of Duloxetine on Amylose based stationary phase", *Journal of chromatographic science, Vol 46, Oct. 2008*
50. Wanjaridb,GaikwadNj,**(2005)** "Reversed Phase HPLC Method for Determination of Glimeperide in Tablet Dosage form".*Journal of Pharmaceutical sciences 67*,253-255.
51. Zahi Mohammad Turabi*, O' hood A. Khatatbeh, Dalal N. Al-Abed*, (2014)," RP-HPLC method development and validation for the simultanious determination of Mebendazole and the two Preservatives Methylparaben and propylparaben in pharmaceutical oral suspension Dosage form", Quality control department, Sama Pharmaceuticals manufacturing Co, Nablus, Palestine, International Journal of Pharmaceutical sciences and drug Research 2014; 6 (1): 70-74.*
52. Zhivkova ZD, Russeva VN. "Stereoselective binding of ketoprofen enantiomers to human serum albumin studied high-performance liquid affinity chromatography." J. Chromatogr. B Biomed. Sci. Appl.714(1998):227-283.

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